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# Small Rodents and Other Mammals Associated with Mountain Meadows as Reservoirs of *Giardia* spp. and *Campylobacter* spp.

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**Sixty-five percent (469 of 722) of the fecal samples collected from small rodents in the central Washington Cascade mountains were positive for *Giardia* spp. Trapping studies showed that microtines of the genus *Microtus* were heavily infected with the parasite. Morphologically the cysts and trophozoites were of the *Giardia duodenalis* type. Small-rodent populations appear to maintain their infection throughout the year. Our data suggest that there is no difference in the percentage of positive animals in areas receiving a lot of human use as opposed to animals in those areas receiving very little or no human use. *Giardia* spp. were also found in elk and beaver fecal samples. *Campylobacter* spp. were recovered infrequently from the small rodents inhabiting alpine meadows. Of 551 specimens cultured, <1% were positive for the bacterium, and the isolates were identified as *Campylobacter coli*. Water voles were susceptible to a human isolate of *Campylobacter jejuni* and shed the bacterium for several weeks. *C. jejuni* was also isolated from a bear fecal sample collected from a protected watershed. Our studies indicate that microtines and possibly other small rodents inhabiting mountain meadows have a potential to act as a reservoir for both *Giardia* spp. and *Campylobacter* spp. Because these animals may carry human pathogens, they should be included in animal surveys designed to assess the health risks associated with mountain watersheds.**

In recent years an apparent increase in human diarrheal disease has been reported among backpackers frequenting high mountain areas and among residents of communities using mountain streams for domestic consumption. In many of these outbreaks *Giardia lamblia* has been reported to be the causative agent (1, 4, 9, 11, 16, 21), but in some instances *Campylobacter jejuni* has been found to be responsible (3, 5, 25). Possible reservoirs of these two disease agents include various wild animal populations. Beavers and muskrats have been reported to be important carriers of *Giardia* spp. (6, 10, 18), and both birds (15, 17, 22) and muskrats (18) have been shown to harbor *C. jejuni*. *Campylobacter* spp. also have been reported in the bank vole (8) and the blue hare (19).

Epidemiological studies conducted during many outbreaks of waterborne diarrheal disease have included surveys of wild animal populations in watersheds and wilderness areas. Animal surveys have generally been restricted to a study of larger animals and have not included a study of microtines and other small rodents as possible reservoirs for these disease agents. Many of these small mammals inhabit moist alpine meadows and stream banks, and their habits lend themselves to the contamination of surface water.

Preliminary studies in our laboratory have shown that water voles (*Microtus richardsoni*) carry *Giardia* spp. (unpublished data), and a more detailed study of these and other small rodents as potential reservoirs of human disease seemed warranted. The present study was undertaken to assess the prevalence of *Giardia* spp. and *Campylobacter* spp. among small-rodent populations inhabiting high mountain meadows. Characteristics of the organisms found in these animals are reported, and the potential for small rodents to act as reservoirs of human disease is discussed. Opportunities to examine other animal populations developed during the present studies, and these findings are included.

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## MATERIALS AND METHODS

**Field studies.** This study was conducted primarily in the central Washington Cascade mountains. Specific study sites consisted of mountain meadows, lakes, and streams. The sites were chosen, in part, by the amount of human use in the area. Areas receiving little or no use as well as areas heavily used were desired to assess the impact of human use on the occurrence of *Giardia* spp. in animal populations. With the assistance of personnel of the U.S. Forest Service, each study site was ranked with regard to level of human use. The categories established were based on use during the summer months of July through September. During other seasons of the year the study sites received lower levels of use. The human use categories established are defined as follows: heavy use, more than 15 people in the area per day; moderate use, 5 to 15 people in the area per day; light use, 1 to 5 people in the area per day; intermittent use, less than 1 person in the area per day; none, sites closed to general public use. The study sites ranged in elevation from 1,600 feet (488 m) to 6,000 feet (1,829 m) above sea level.

In addition to samples collected from sites in the central Washington Cascade mountains, a small number of samples were also obtained from the Rattlesnake Creek drainage near Missoula, Mont. These samples were obtained immediately after an outbreak of giardiasis in the Missoula area.

A summary of the various study sites and their characteristics is included in Table 1.

**Samples.** Samples were collected over a 24-month period from 1983 through 1985. Both fresh fecal samples and live-trapped animals were examined in this study. Fecal pellets from small rodents (*Microtus richardsoni*, *Microtus longicaudus*, *Zapus princeps*, *Peromyscus maniculatis*, etc.) were collected from stream banks and rodent runways within the study sites. Only those fecal pellets which were soft and moist and which appeared to be recently deposited were collected. Based on the appearance of the fecal pellets,

TABLE 1. Occurrence of *Giardia* cysts in fecal pellets from small rodents

| Location | Elevation (ft)[a] | Human use (summer mos)[b] | No. sampled | No. positive | % Positive |
|---|---|---|---|---|---|
| Protected watershed | | | | | |
| Meadow 100–300 | 1,600 | None | 3 | 0 | 0 |
| Meadow 610–620 | 2,400 | None | 48 | 3 | 6 |
| Meadow 700 | 2,600 | None | 53 | 36 | 68 |
| Meadow 650 | 3,300 | None | 98 | 80 | 82 |
| Cooper Lake | 2,800 | Heavy | 68 | 36 | 53 |
| Upper Yakima River | 2,800 | Moderate | 12 | 12 | 100 |
| Cooper Pass | 3,000 | Intermittent | 52 | 33 | 63 |
| Lizard Lake | 3,200 | Heavy | 24 | 20 | 83 |
| Naneum Meadow | 5,400 | Light | 131 | 97 | 74 |
| Haney Meadow | 5,500 | Moderate | 24 | 15 | 63 |
| Bean Creek | 5,500 | Intermitent | 34 | 24 | 71 |
| Hereford Meadow | 5,800 | Light | 138 | 87 | 63 |
| Longs Pass | 6,000 | Intermittent | 6 | 3 | 50 |
| Rattlesnake Creek drainage (Montana) | 3,500–4,700 | Variable | 31 | 23 | 74 |
| Totals | | | 722 | 469 | 65 |

[a] 1 ft = 30.48 cm.

[b] Human use categories: heavy, more than 15 people per day; moderate, 5 to 15 people per day; light, 1 to 5 people per day; intermittent, less than 1 person per day; variable, human use varies from intermittent to heavy within the watershed.

the majority of the samples most likely were from rodents of the genus *Microtus*. Each sample consisted of a group of pellets from what appeared to be a deposit from a single animal. Fecal samples from other animals studied were obtained from various locations throughout the sites. Trapping studies were designed to assess the occurrence of *Giardia* spp. in various populations of small rodents. Sherman live traps were used to obtain animals for study in this portion of the investigation.

Fecal samples were transported to the laboratory on ice and examined as soon as possible. Nearly all the samples screened for *Campylobacter* spp. were processed within 6 h of collection. Those specimens which could not be processed within this time were inoculated into Amies transport medium (Difco Laboratories, Detroit, Mich.) and processed as soon as the samples reached the laboratory. All fecal specimens examined for cysts of *Giardia* spp. were analyzed within 48 h after collection.

Fecal pellets from all live-trapped animals were examined for cysts of *Giardia* spp. In addition, the small intestinal mucosa of all animals that died of exposure in the traps and all live-trapped species other than the water vole (*M. richardsoni*) was examined for *Giardia* trophozoites. Water voles captured in this study were added to our animal colony.

**Giardia detection.** Fecal specimens were screened for *Giardia* spp. by examining trichrome-stained direct smears of fecal pellets and by a membrane filter procedure (23). Slides were screened at ×400 magnification, and cysts of *Giardia* spp. were confirmed at ×1,000 magnification. Internal characteristics used to identify the cysts were those described by Schaefer and Rice (20) and included two to four nuclei, median bodies, and axonemes. The number of cysts per gram of fecal material was quantified by a membrane filter procedure (23). Trophozoites of *Giardia* spp. in live-trapped animals were detected by examining scrapings of the duodenal mucosa by phase-contrast microscopy.

***Campylobacter* isolation and identification.** Methods used to detect and identify *Campylobacter* spp. in fecal pellets have been described previously (18).

**Susceptibility of water voles to *C. jejuni* isolated from humans.** *C. jejuni*-free water voles (1 to 2 months old) from our vole colony were used. Before inoculation, both control and experimental animals were screened for *Campylobacter* spp. by culturing pooled fecal pellets from each animal as previously described (18). Inoculation was achieved by the administration of a dilute broth culture of *C. jejuni* into the esophagus of lightly anesthetized animals which had been deprived of water for 4 to 6 h. Metofane (Pitman-Moore, Inc.) was used as an anesthetic. After inoculation, fresh fecal specimens were cultured for the bacterium by procedures described previously (18).

The strain of *C. jejuni* used in these experiments was obtained from the clinical laboratory at the University of Washington Hospital. The strain was a fresh isolate from a case of *Campylobacter* enteritis in a human. This isolate was maintained at −70°C before use. Cultures used in the susceptibility studies were grown in brucella broth (Difco Laboratories, Detroit, Mich.) at 42°C under microaerobic conditions. Dilutions of the culture were prepared in phosphate-buffered water and used immediately. The doses administered were determined by plate counts.

## RESULTS

**Occurrence of *Giardia* and *Campylobacter* spp. in small-rodent populations.** A total of 722 fecal samples collected from the various study sites were examined for *Giardia* spp. As shown in Table 1, 469 (65%) of these were infected with the parasite. There appeared to be no correlation between the amount of human use occurring in the area and the presence of infected animals. *Giardia* spp.-infected animals were found both in areas receiving heavy human use and in those locations having little or no human use. In one study site (meadow 650), 82% of the specimens examined contained *Giardia* spp. This site is in a protected watershed which has been closed to general public use for over 80 years. This suggests that a large proportion of the rodent population in the area is infected with the parasite.

The study sites selected for this study ranged in elevation from 1,600 feet to over 6,000 feet above sea level. Infected animals were found at all these locations with the exception of the site located at 1,600 feet where the sample size was small. Thus, small-rodent populations infected with *Giardia* spp. appear to be widely distributed in the central Washington Cascade mountains.

Animal trapping studies were conducted at a number of the study sites in an effort to determine the species of rodents which harbor *Giardia* spp. Animals captured included two species of voles (*M. richardsoni* and *M. longicaudus*), jumping mice (*Z. princeps*), deer mice (*P. maniculata*), shrews (*Sorex vagrans*), and weasels (*Mustela erminea*) (Table 2). Only the *Microtus* spp. were infected with *Giardia* spp.; however, the number of other animals trapped was small, and the results may not accurately represent the incidence of *Giardia* spp. in other rodent populations. Forty-one voles were captured, and all were infected with the parasite. The trophozoites observed in the intestinal mucosa of the voles showed elongated median bodies and did not resemble *Giardia muris*, the *Giardia* species commonly described in laboratory mice and rats.

The quantity of *Giardia* cysts in fresh fecal pellets from 23 recently trapped microtines was determined. The mean cyst density ranged from $3.4 \times 10^3$ to $3.7 \times 10^4$/g. The range in

TABLE 2. Occurrence of *Giardia* spp. in live-trapped animals

| Location | Elevation (ft) | Common name | No. of positive/ total |
|---|---|---|---|
| Protected watershed (meadow 650) | 3,300 | Water vole (*M. richardsoni*) | 1/1 |
| | | Longtail vole (*M. longicaudus*) | 4/4 |
| Cooper Pass | 3,000 | Water vole (*M. richardsoni*) | 2/2 |
| Cooper Lake | 2,800 | Water vole (*M. richardsoni*) | 3/3 |
| | | Deer mouse (*P. maniculatus*) | 0/3 |
| Naneum Meadows | 5,400 | Longtail vole (*M. longicaudus*) | 14/14 |
| | | Deer mouse (*P. maniculatus*) | 0/2 |
| | | Western jumping mouse (*Z. princeps*) | 0/4 |
| | | Vagrant shrew (*S. vagrans*) | 0/1 |
| | | Shorttail weasel (*M. erminea*) | 0/1 |
| Hereford Meadows | 5,800 | Water vole (*M. richardsoni*) | 7/7 |
| Bean Creek | 5,500 | Water vole (*M. richardsoni*) | 7/7 |
| Fourth Creek | 5,800 | Water vole (*M. richardsoni*) | 3/3 |

number of cysts per gram of fecal material varied from approximately 1,000 to 50,000.

Fresh fecal pellets from the trapped animals as well as 551 of the rodent fecal pellet samples collected from the study sites were also examined for *Campylobacter* spp. The bacterium was isolated from only four of the fecal pellet groups collected, and all these came from the Naneum Meadow study site. The bacterium was not isolated from any of the trapped animals. The isolates obtained from the rodent fecal pellets were identical and were consistent with *Campylobacter coli*. All grew at 42°C, were sensitive to nalidixic acid, and failed to hydrolyze sodium hippurate. It is of interest to note that isolates of *C. coli* were also obtained from mud samples collected from stream beds in Naneum Meadow.

**Incidence of *Giardia* and *Campylobacter* spp. in fecal samples from larger animals.** In addition to the fecal samples from the small-rodent populations, fecal samples from a variety of other animals were also collected at the study sites and examined for *Giardia* spp. The parasite was detected in both elk and beaver samples but not in rabbit or bear samples (Table 3). Of 115 elk pellet groups examined, 2 contained cysts of *Giardia* spp. The cyst density in one of these samples was $1.4 \times 10^5$/g of fecal material. It should be noted that the elk samples containing *Giardia* spp. were collected in a protected watershed that had been closed to public use for over 80 years.

The beaver samples containing *Giardia* spp. all were collected from beaver dams in the Manastash Creek drainage. These dams are located in an area that receives only infrequent human use in summer and no human use in winter. Fecal samples were collected from these sites during the period April 1985 through December 1985, and scats infected with *Giardia* spp. were found throughout this period. Of the 272 samples examined from this location, 76 (28%) contained the parasite.

Beaver, elk, rabbit, and bear fecal samples were also screened for *Campylobacter* spp. A total of 199 samples from these sources were cultured, and the bacterium was isolated from one bear sample (Table 4). This sample was collected from within a protected watershed. The biochemical characteristics of the isolate were consistent with those of *C. jejuni*. The organism hydrolyzed sodium hippurate, was sensitive to nalidixic acid, and grew at 42°C.

**Morphological features of *Giardia* spp. occurring in water voles and elk.** The cysts of *Giardia* spp. from the water vole showed two to four nuclei usually located near one end, distinct axonemes, and elongate dark-staining bodies located

TABLE 3. Occurrence of *Giardia* cysts in fecal pellets from mammals other than small rodents

| Location | Elevation (ft) | Human use[a] | No. of positive specimens/total | | | |
|---|---|---|---|---|---|---|
| | | | Elk | Beaver | Rabbit | Bear |
| Protected watershed | | | | | | |
| Meadow 100–300 | 1,600 | None | 2/66 | 0/5 | 0/3 | 0/3 |
| Meadow 610–620 | 2,400 | None | | | 0/9 | |
| Meadow 700 | 2,600 | None | 0/9 | | | |
| | | | | | 0/3 | |
| Meadow 650 | 3,300 | None | 0/2 | 0/1 | | 0/3 |
| Manastash Creek | 1,700 | Intermittent | 0/6 | 76/272 | | |
| Teanaway | 1,800 | Light | | 0/3 | | |
| Circle LDS Ranch | 2,500 | Heavy | | 0/22 | | |
| Upper Yakima River | 2,800 | Moderate | | 0/10 | | |
| Naneum Meadow | 5,400 | Light | 0/26 | | | |
| Hereford Meadow | 5,800 | Light | 0/6 | | | |

[a] See Table 1, footnote b, for human use categories.

TABLE 4. Occurrence of *Campylobacter* spp. in fecal samples from mammals other than small rodents

| Location | Elevation (ft) | Human use[a] (summer mos) | No. of positive specimens/total | | | |
|---|---|---|---|---|---|---|
| | | | Elk | Beaver | Rabbit | Bear |
| Protected watershed | | | | | | |
| Meadow 100–300 | 1,600 | None | 0/66 | 0/12 | 0/3 | 0/3 |
| Meadow 610–620 | 2,400 | None | | 0/9 | | |
| Meadow 700 | 2,600 | None | 0/9 | | | |
| Meadow 650 | 3,300 | None | 0/2 | 0/1 | | 1/3 |
| Manastash Creek | 1,700 | Intermittent | 0/6 | 0/40 | | |
| Circle LDS Ranch | 2,500 | Heavy | | 0/9 | | |
| Rattlesnake Creek (Montana) | 3,500–4,700 | Variable | | 0/4 | | |
| Naneum Meadows | 5,400 | Light | 0/26 | | | |
| Hereford Meadows | 5,800 | Light | 0/6 | | | |

[a] See Table 1, footnote *b*, for human use categories.

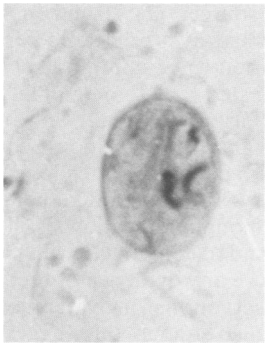

FIG. 1. Trichrome-stained *Giardia* cyst from water vole (magnification, ×1,860).

transversely in the organism (Fig. 1). These dark-staining structures could be either median bodies or remnants of the adhesive disk. The dimensions of the cysts were compared with those of cysts from beavers, muskrats, elk, and humans (Table 5). The average dimension of 36 water vole cysts was 6.5 by 11.0 μm. The mean length and width of cysts from beavers and humans were slightly larger than those from muskrats and water voles, but there is overlap in the dimensions of cysts from all four sources.

*Giardia* trophozoites observed from the small intestine of the water vole were teardrop shaped and exhibited distinct elongate median bodies similar to those found in trophozoites from humans, beavers, and muskrats (Fig. 2).

The *Giardia* cysts from elk are shown in Fig. 3. The cysts were oval, and the cytoplasm was closely applied to the cyst wall. The average width and length of 50 cysts measured under oil immersion was 7.5 by 11.0 μm with a range of 7.0 to 8.0 μm by 9.0 to 13.0 μm. Four large nuclei were observed in most of the cysts, and in the majority, these nuclei were located near one end. Well-defined axonemes were evident within the cysts, and occasionally elongate dark-staining bodies (median bodies or remnants of the adhesive disk) were noted. Morphologically, the cysts from elk were similar in appearance to cysts observed in our laboratory from humans, beavers, muskrats, and water voles.

**Susceptibility of water voles to *C. jejuni* of human origin.** *C. jejuni* has been shown to be prevalent in muskrats (18), and bank voles are known to carry *Campylobacter* spp. (8). Since both of these animals are microtines and related to the voles studied in the present investigation, it was of interest to determine whether the water vole could be infected with *C. jejuni* and possibly serve as a reservoir for this bacterium. The strain of *C. jejuni* used for these experiments was isolated from a case of campylobacteriosis in a human. In

TABLE 5. Dimensions of *Giardia* cysts from humans, beavers, muskrats, water voles, and elk[a]

| Source | No. of cysts measured[b] | Mean (μm) | | Range (μm) | |
|---|---|---|---|---|---|
| | | Length | Width | Length | Width |
| Human | 26 | 12.0 | 7.5 | 9.0–13.0 | 6.0–9.0 |
| Beaver | 21 | 13.0 | 7.0 | 12.0–14.0 | 7.0–8.0 |
| Muskrat | 28 | 11.5 | 7.0 | 9.0–13.0 | 5.0–8.0 |
| Water vole | 36 | 11.0 | 6.5 | 11.0–13.0 | 6.0–7.0 |
| Elk | 50 | 11.0 | 7.5 | 9.0–13.0 | 7.0–8.0 |

[a] Cyst measurements were made at ×1,000 magnification on hot-Schaudinn-fixed, trichrome-stained fecal smears with an ocular micrometer.
[b] Cysts measured were obtained from multiple fecal specimens.

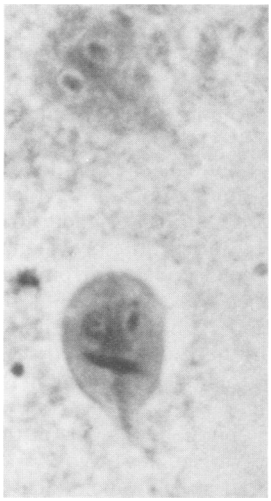

FIG. 2. Trichrome stain of *Giardia* trophozoites from the intestinal mucosa of the water vole (magnification, ×1,860).

preliminary experiments with 9- to 12-month-old voles it was established that these animals could be infected by the oral administration of large doses ($10^6$ to $10^7$ cells) of a broth culture of the organism (unpublished data). These animals shed the bacterium in their fecal pellets for 3 to 5 weeks. Using newly weaned animals (1- to 2-month-old voles), it was possible to achieve infection in some instances at lower doses of the bacterium. The results of the experiments with newly weaned animals (Table 6) indicate that doses as low as $8 \times 10^4$ cells are able to infect water voles and that these animals shed the bacterium for up to 9 weeks.

## DISCUSSION

Little attention has been given to the occurrence of *Giardia* spp. in water voles (*M. richardsoni*) and other small-rodent populations inhabiting the high mountain areas of the central Washington Cascades. Frost et al. (10) examined a variety of animals trapped throughout the state of Washington, and both beavers and muskrats were found to be infected with the parasite. These studies, however, did not include an examination of small-rodent populations. Our study was concerned primarily with the prevalence of *Giardia* spp. in small-rodent populations inhabiting moun-

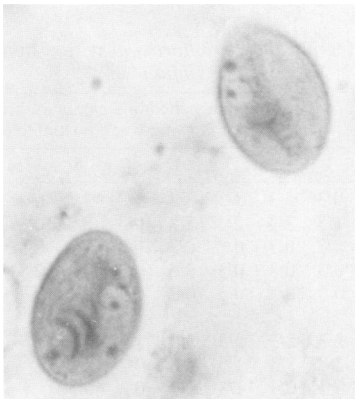

FIG. 3. Trichrome-stained *Giardia* cysts from elk (magnification, ×1,860).

tain meadows in central Washington and showed that the parasite is present in a large proportion of these mammals. Of the small-rodent fecal samples examined, 65% were positive for *Giardia* spp. The prevalence of infection among the small rodents examined in our study was only slightly lower than the levels reported by other investigators who have studied similar animals. Grant and Woo (12) reported a level of infection among meadow voles and deer mice collected in southern Ontario, Canada, to be approximately 98%. Wallis et al. (26) found the prevalence of *Giardia* spp. in three species of voles collected from Banff National Park to be 77%. Deer mice were also studied by these investigators and were found to have a much lower incidence of infection (10%). In his studies in the Sierra Nevada in California, Suk (24) found *Giardia* spp. in the mountain voles and other mammals.

Trapping studies conducted as part of the present investigation showed that two species of voles (*M. richardsoni* and *M. longicaudus*) had a high incidence of infection with *Giardia* spp. Although only a small number of small rodents of other species were trapped in our study, none were infected with the parasite. Our findings are similar to those of Wallis et al. (26) in that their investigation found a much higher incidence of *Giardia* spp. in voles than in other small rodents. In contrast, Grant and Woo (12) found *Giardia* spp. to be prevalent in both meadow voles (*Microtus pennsylvanicus*) and deer mice (*P. maniculatis*). In our studies, the water vole (*M. richardsoni*) and the long-tailed vole (*M. longicaudus*) were the predominant species of small rodent infected with *Giardia* spp. Both of these voles inhabit mountain meadows at elevations well above those inhabited by beavers and muskrats.

In their study, Wallis et al. (26) presumed the *Giardia* spp. found in the microtines examined to be *G. muris*. This is the species of organism commonly reported in laboratory rats and mice. The trophozoites observed in both the water vole and the long-tailed vole in our study showed characteristics different from those of *G. muris*. The morphological features of these trophozoites were of the *Giardia duodenalis* type, which is characterized by a teardrop-shaped cell and an elongate median body (Fig. 2). In contrast, the trophozoites of *G. muris* are nearly round and have a round median body. Since the *Giardia* species found in the water vole is of the *G. duodenalis* type it may infect humans. Further similarities between the *Giardia* spp. found in water voles and that which infects humans are suggested by the intermittent pattern of cyst release in these animals. This pattern of cyst release has been described in gerbils infected with *Giardia* spp. from human sources (2, 7) and in naturally infected voles (13). In contrast, cyst release in gerbils infected with *G. muris* was reported to be continuous (7).

TABLE 6. Infection of *M. richardsoni* with a human isolate of *C. jejuni*

| Expt | Dose | No. of animals shedding campylobacter/total at the following week postinfection: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | $5 \times 10^7$ | 0/3 | 3/3 | 3/3 | 3/3 | 1/3 | 3/3 | 0/3 | 2/3 | 2/3 | 0/3 |
| | $5 \times 10^6$ | 1/3 | 2/3 | 2/3 | 2/3 | 0/3 | 0/3 | 0/3 | | | |
| | $5 \times 10^5$ | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | | | |
| | $5 \times 10^4$ | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | | | |
| | $5 \times 10^3$ | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | | | |
| 2 | $8 \times 10^7$ | 2/3 | 2/3 | 2/3 | 3/3 | 1/3 | 0/3 | | | | |
| | $8 \times 10^6$ | 3/3 | 3/3 | 1/3 | 2/3 | 0/2 | 0/2 | | | | |
| | $8 \times 10^5$ | 0/3 | 0/3 | 1/3 | 1/3 | 0/3 | 0/3 | | | | |
| | $8 \times 10^4$ | 1/3 | 1/3 | 1/3 | 1/3 | 2/3 | 1/3 | | | | |
| | $8 \times 10^3$ | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | | | | |

It has been suggested that some animal species lose their *Giardia* infection during the winter and become reinfected each summer (6). Our findings indicate that small-rodent populations inhabiting mountain meadows retain their infection throughout the year. Fresh rodent pellets collected early in the spring from mountain meadows still partially covered with snow were found to contain *Giardia* spp. These samples were collected before any evidence of recent human activity was apparent. Observations on water voles maintained in our animal colony also support this suggestion (unpublished data). These animals may become infected with *Giardia* spp. shortly after birth if the mother is infected with the parasite, and many of the animals retain their infection throughout their lives even when held under isolated conditions.

Some researchers (6) have suggested that sewage plays an important role in maintaining *Giardia* spp. infections in aquatic animals. Our findings do not totally support this contention. In our study, fecal pellets and live-trapped animals collected from a protected watershed closed to general public use for over 80 years were positive for *Giardia* spp. The prevalence of infection in these samples was as high as that occurring in areas receiving heavy human use. These results suggest that neither sewage nor human activity in an area is necessary for small rodents inhabiting mountain meadows to become infected with *Giardia* spp. Similar observations were noted by Frost et al. (10) in their studies on commercially trapped animals.

During this investigation an outbreak of giardiasis occurred in Missoula, Mont. This outbreak provided an opportunity for us to study small-rodent populations in an area outside the central Washington Cascade mountains which had been associated with a recent outbreak of giardiasis. Fecal samples collected from small-rodent runways within the watershed used as the water supply for the city of Missoula had a high incidence of *Giardia* spp. Seventy-four percent of the samples examined from the area were positive for the parasite. It cannot be established for certain, however, whether these small rodents played a role in this outbreak of disease.

In conjunction with studies on small-rodent populations, fecal material from other animals was collected and examined for *Giardia* spp. Both beaver and elk fecal material containing *Giardia* cysts were observed. Beavers from various locations in the state of Washington have been reported to be infected with *Giardia* spp. (10). However, to our knowledge *Giardia* spp. have not been previously described in elk. Davies and Hibler (6), Johnston (14), and Wallis et al. (26) examined fecal samples from elk, and none were found to be infected with the parasite. Davies and Hibler (6) also attempted to infect elk with *Giardia* spp. from a human source, and no evidence of infection was obtained. The *Giardia* cysts which we observed in elk fecal material were morphologically similar to cysts observed in other animal species studied in this investigation. While it cannot be concluded that elk represent a reservoir of sylvatic giardiasis, further investigations on these animals are warranted.

A variety of wild animal species have been reported to act as reservoirs for *C. jejuni* (8, 15, 17, 19, 22). Among wild rodent populations, the bacterium has been detected in bank voles (8) and muskrats (18). Our studies showed a very low incidence of *Campylobacter* spp. in small-rodent populations in the central Washington Cascade mountains. The

bacterium was isolated only from a small number of fecal samples collected from Naneum Meadow and not from any live-trapped animals. As a result, the species of rodent carrying the bacterium could not be determined. It is of interest, however, that it was possible to infect water voles (*M. richardsoni*) with an isolate of *C. jejuni* from a human infection. Although rather high doses of the bacterium were required to achieve infection, the rodents shed the bacterium for several weeks. *C. jejuni* can thus exist in the intestinal tract of these voles, and hence they have the potential to act as a reservoir of the bacterium in high mountain areas.

*C. jejuni* also was isolated from a bear sample collected from a protected watershed. It is not clear from our studies how bears acquire the infection; however, these animals should be included among wild animal species capable of acting as a reservoir for the bacterium.

Our studies suggest that small rodents inhabiting alpine meadows and particularly voles of the genus *Microtus* act as reservoirs for *Giardia* spp. and under proper circumstances for *C. jejuni*. The habits of these animals are such that disease-producing agents carried by these rodents could readily become waterborne. Both the water vole (*M. richardsoni*) and the long-tailed vole (*M. longicaudus*) are found in mountain meadows at relatively high elevations, and they tend to defecate along stream banks and in running water. As a result, any disease-producing agent carried in the intestinal tracts of these rodents could readily become disseminated. These animals should thus be considered as a potential reservoir of human disease agents, and epidemiological investigations on waterborne outbreaks of disease in alpine areas as well as surveys on potential health risks in these areas should include a study of small-rodent populations in addition to the larger mammals.

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